HALF-LIFE OF RABBIT HEART MITOCHONDRIA IN EXPERIMENTAL ALLERGIC MYOCARDIAL DAMAGE

M. D. Grozdova, L. K. Starostina, I. L. Sharlova

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The half-life ($T_{1/2}$) of the proteins and cytochorme c of rabbit myocardial mitochondria was determined by the use of glycine-1-C^{14} and aminolevulinic acid-H^3, respectively, as precursors. $T_{1/2}$ of cytochrome c, calculated from the dynamics of the decrease in content of the label in the course of several weeks of investigation, was 11.8 days and $T_{1/2}$ of the mitochondrial proteins 14.4 days. The degree of reutilization of glycine-C^{14} in structural metabolism was about 20%. No change was found in the rate of degradation of the mitochondria in rabbits with allergic myocardial damage.

KEY WORDS: myocardium; mitochondria – rate of breakdown; allergic damage.

Mitochondria (except the components of the outer membrane and certain soluble enzymes) are replaced as a single entity [7, 9]. It is not yet clear whether degradation of the mitochondria takes place through phagocytosis by lysosomes [12] or through gradual decay. This accounts for the interest in the study of degradation of mitochondria in pathological states, often connected with an increase in the number of lysosomes and the labilization of their structure.

To determine the half-life ($T_{1/2}$) of mitochondria, it is convenient to use labeled 5-aminolevulinic acid (5-ALA). When taken into the body, 5-ALA is incorporated into the prosthetic group of the mitochondrial cytochromes (incorporation of label into the apoenzyme is not significant [7]), after which, as the mitochondria decay, it is excreted as bilirubin and is not reutilized in structural metabolism of the tissues [7, 9]. Amino acids as precursors of mitochondrial proteins when liberated during protein breakdown can be reutilized, thus distorting the true rate of breakdown. Comparison of data obtained by the use of a nonreutilizable precursor and values obtained by the use of an amino acid reflects the degree of reutilization of the usable compound [14].

The object of this investigation was to determine $T_{1/2}$ for rabbit myocardial mitochondria under normal conditions and in experimental allergic myocardial damage.

EXPERIMENTAL METHOD

Male rabbits weighing 2.2-2.8 kg were used. Glycine-1-C^{14} (V/O "izotop," USSR, specific activity 6.2 mCi/mmole) and 5-ALA-H^3 (Radiochemical Centre, Amersham, England, specific activity 2 Ci/mmole) were injected intravenously in doses of 50 μCi and 450 μCi/kg respectively. Allergic myocardial damage was induced by repeated injection of normal horse serum into the rabbits [1]. The 5-ALA-H^3 was injected 10 days, and glycine-1-C^{14} seven days, after the appearance of pathological changes. The control animals received injections of physiological saline instead of horse serum. The heart was removed from the animals under ether anesthesia, at various times after injection of the isotope or physiological saline, and repeatedly washed with 0.15 M KCl solution to remove the blood. The minced tissue of the ventricles was

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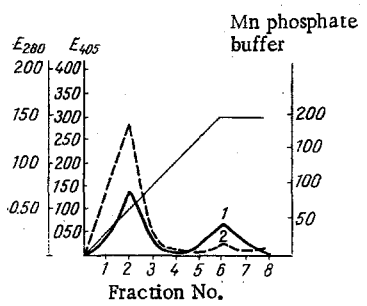

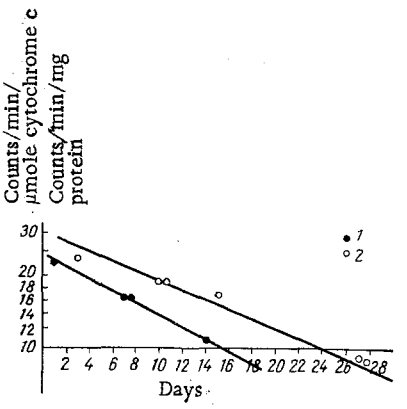

Fig. 1. Chromatography of eluate of mitochondria on CM-cellulose in phosphate buffer gradient (pH 6.8). Dimensions of column 0.5 × 20 cm. Rate of elution 3 ml/h. Cytochrome c eluted with 0.2 M phosphate buffer: 1) extinction at 405 nm; 2) extinction at 280 nm.

Fig. 2. Dynamics of fall of specific radioactivity of cytochrome c and mitochondrial proteins from normal heart: 1) cytochrome c labeled with 5-ALA-H^3; 2) mitochondrial proteins labeled with glycine-1-C^{14} (curve calculated by method of least squares). Each point represents results of experiment on two animals.

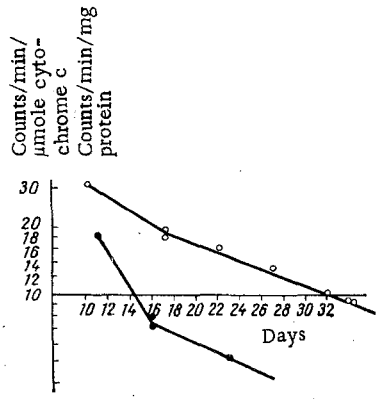

Fig. 3. Dynamics of fall in specific radioactivity of cytochrome c and mitochondrial proteins of heart with experimental allergic damage. Abscissa, time (days) from beginning of development of pathological process in heart. Remainder of legend as in Fig. 2.

homogenized with a Teflon pestle in 0.25 M sucrose solution with 0.01 M tris buffer, pH 7.4, and the mitochondria were sedimented and reprecipitated twice by centrifugation within the range 600-9000 g. Cytochrome c was extracted from the mitochondria with 0.15 M KCl solution [11] and then purified on two columns of CM-cellulose [13]; it was then determined quantitatively from the extinction at 405 nm, using a coefficient of molar extinction of $93.6 \cdot 10^3$ [16]. Mitochondria containing glycine-1-C^{14} and the tissue homogenate in the experiments with ALA were each treated with TCA in a final concentration of 5%. The precipitated proteins were washed three times with 2.5% TCA, defatted and dried with alcohol and ether, and hydrolyzed in 0.3 N KOH solution; the digest was used to determine radioactivity by means of a liquid scintillation counter, the samples for counting containing not more than 1-2 mg protein. Carbon-14 was determined by the method of Wannemacher et al. [18], and tritium in Bray's mixture [5]. The radioactivity of cytochrome c (fractions removed from the column) was determined in a Triton X-100–toluene–water mixture (ratio 4 : 3 : 3 selected experimentally); the toluene contained 2,5-diphenyloxazole and 1,4-di[2-(5-phenyl)-oxazolyl]benzene (POPOP) in quantities of 12 and 300 ml/liter respectively [8]. The efficiency was determined by channel ratio method. Protein was determined by the method of Lowry et al. [15].

EXPERIMENTAL RESULTS AND DISCUSSION

The rate of turnover of the prosthetic group of cytochrome c and the apoenzyme is evidently identical [3], which means that labeled ALA, incorporated into heme during its biosynthesis, can be used as the cytochrome precursor. The elution curve from the first CM-cellulose column after application of mitochondrial extract is shown in Fig. 1. Cytochrome c was eluted by 0.2 M phosphate buffer (fraction No. 6). The ratio between extinctions at 405 and 280 nm for this fraction varied between 3.1 and 4.1, the theoretical value being 4.03. Rechromatography of the cytochrome c fraction on a CM-cellulose microcolumn yielded a more purified preparation. The spectrum of the fraction recorded between 250 and 600 nm corresponded closely to the spectrum of oxidized cytochrome c [16].

From the fall in the specific radioactivity of cytochrome c during the 14 days of investigation, plotted on a semilogarithmic scale, the value of $T_{1/2}$ for cytochrome c was calculated to be 11.8 days (Fig. 2). By

determining $T_{1/2}$ with the aid of glycine-C^{14}, a somewhat higher value was obtained, namely 14.4 days. It can be calculated [14] that the degree of reutilization of this amino acid is 22%.

The value of $T_{1/2}$ for cytochrome c, which according to our calculations was 11.8 days, suggests that with respect to this index the mitochondria of the rabbit myocardium do not differ significantly from the mitochondria of rat liver, $T_{1/2}$ for which lies between the limits of 6 and 12 days.

According to information in the literature, $T_{1/2}$ for rat liver and heart mitochondria is identical [3, 9]. Other workers [12, 17], however, found differences between $T_{1/2}$ for mitochondria from the heart and liver.

Evidence is accumulating in the literature to show that the normal decay of mitochondria may be disturbed under various conditions: acceleration of this process in some organs has been observed in hypoxia [4], after administration of thyroxine [10], and during adaptation to cold [6].

Electron-microscopic investigations of the myocardium after allergic damage [2] have revealed activation of the lysosomal system 7-20 days after the appearance of pathological changes, and this could modify $T_{1/2}$ of the tissue protein structures. It must be remembered that on the 10th-20th day of development of pathological changes in the heart, mitochondrial protein synthesis is activated [1]. Under those conditions the change in specific radioactivity reflects the rate of dilution of the label as a result of synthesis, whereas the decrease in the content of label in the organ as a whole must serve as index of the actual rate of breakdown [14].

Determination of the dynamics of elmimnation of both ALA-H^3 and glycine-C^{14} (Fig. 3) in fact yielded a curve with an inflection: to begin with there was a rapid decrease in the content of the label, which could be connected with activation of synthesis; the content of label then fell at the same rate as normally ($P > 0.05$). $T_{1/2}$ determined with the aid of glycine-C^{14} was 16.2 days and with the aid of ALA-H^3 12.2 days. These results indicate that mitochondria synthesized under pathological conditions have a nearly normal half-life.

LITERATURE CITED

1. M. D. Grozdova and L. K. Starostina, Vopr. Med. Khimii, No. 4, 403 (1973).
2. M. D. Grozdova, L. K. Starostina, and P. Ya. Mul'diyarov, in: Biophysical Basis of the Pathological State of the Muscles and Energy Supply to the Contractile System (Proceedings of a Conference) [in Russian], Tbilisi (1973), p. 51.
3. V. Ashenbrenner, R. Druyan, R. Albin, et al., Biochem. J., 119, 157 (1970).
4. V. Ashenbrenner, R. Albin, and A. F. Cuttileta, Am. J. Physiol., 221, 1418 (1971).
5. G. A. Bray, Analyt. Biochem., 1, 279 (1960).
6. L. Bukowiecki and J. Himms-Hagen, Canad. J. Physiol. Pharmacol., 49, 1015 (1971).
7. R. Druyan, B. de Bernard, and M. Rabinowitz, J. Biol. Chem., 244, 5874 (1969).
8. B. W. Fox, Internat. J. Appl. Radiat., 19, 717 (1968).
9. N. J. Gross, G. S. Getz, and M. Rabinovitz, J. Biol. Chem., 244, 1552 (1969).
10. N. J. Gross, J. Cell Biol., 48, 29 (1971).
11. E. E. Jacob and D. R. Sanadi, J. Biol. Chem., 235, 531 (1960).
12. B. Kadenbach, Biochim. Biophys. Acta, 186, 399 (1969).
13. B. Kadenbach and P. F. Urban, Z. Analyt. Chem., 243, 542 (1968).
14. A. L. Koch, J. Theoret. Biol., 3, 283 (1962).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
16. E. Margoliash and N. Frohwirt, Biochem. J., 71, 570 (1959).
17. R. A. Menzies and P. H. Gold, J. Biol. Chem., 246, 2425 (1971).
18. R. W. Wannemacher, W. L. Banks, and W. H. Wunner, Analyt. Biochem., 11, 320 (1965).